arguments below, Applicants respectfully request reconsideration.

Double Patenting

Applicants have cancelled claims 14, 17, 19 and 20. Applicants had believed that these claims have been cancelled once before in the specification and did not need to be cancelled again. Applicants intend to prosecute claims 24 - 27 and thank the Examiner for the opportunity to clarify this point.

Specification

Applicants have adopted the Examiner's suggested title.

Applicants have enclosed a copy of the Downs and Tempkin paper (now combined). This paper was not published until after the filing date of the above-identified application. Applicants have amended the specification to remove reference to this paper but have enclosed a copy for the Examiner's convenience as much of the description in the specification is replicated in the paper.

Applicants have amended the first paragraph of the specification as the Examiner has suggested.

Claim Objection

Applicant has amended claim 27 as the Examiner has suggested.

§ 112 Rejection

Claims 24 - 27 are rejected under 35 U.S.C. § 112. Applicants first note that claims 24 and 25 have been replaced with new claims 28 and 29, added to replicate the Examiner's language in page 4 of the Office Action, paragraph 1 at (2) and (3).

Applicants first address the Examiner's point at the second paragraph of page 5. Applicants believe the Examiner to require Applicants to more clearly specify which aspect of vasculogenesis is being observed. Applicants point to Fig. 12 as an example of a visual examination of the morphology of the time-course of vasculization in plated head-fold stage allantoises. Applicants have replaced claim 24 with claim 29 and claim 25 with claim 28 to clarify that Applicants are observing the vascularization of the allantoises, as described at page 88 and 89 of the specification and Fig. 12. Note that Applicants have described the appearance of criss-crossing vascular channels as an indication of the appropriate progress of vascularization.

At page 6, first paragraph the Office Action requests that "if the claims require transplanting donor tissue into recipient embryos, the claims should be limited to histocompatible allogenic or syngenic transplantation . . . ." Applicants note that their claims do not require transplanting donor tissue into recipient embryos.

The second full paragraph of page 6 asks that Applicants specify that a comparison step be added to the claim. New claims 28 and 29 add such a step.

At the bottom of this paragraph (top of page 7) the Office Action asks for clarification of the method used to determine if a compound affects vasculogenesis. Applicants have provided this support in the discussion above.

In the first paragraph of page 7, the Office Action has questioned the use of the term "detrimental." Applicants have rewritten the claims to specify that one is observing the effect of the compound on vascularization and have cancelled claim 26.

All claims are rejected under 35 U.S.C. § 112, second paragraph as being indefinite. Claims 14 and 24 are rejected as indefinite "because the metes and bounds of what Applicants consider an 'isolated allantois culture' cannot be determined." Applicants note that new claims 28 and 28 are drawn to "isolating allantois tissue."

All claims are rejected as indefinite because "the metes and bounds of the phrase 'observing vasculogenesis' cannot be determined." Applicants note that claims 28 and 29 are now drawn to observing "vascularization of allantois tissue." The Examiner has noted that "while Applicants' definition states vasculogenesis includes

differentiation of mesodermal cells into angioblasts, the specification does not define that this is the beginning of vasculogenesis." Applicants have clarified the claim by describing observation of "vascularization."

The Office Action notes that "Applicants teach the allantois grows and contacts the chorion prior to becoming the umbilical chord and is essential to form the umbilical chord. It is unclear whether the process of the allantois growing and contacting the chorion is included in 'vasculogenesis' of the umbilical chord." Applicants clarify that contact with the chorion is not included in vasculogenesis or vascularization.

The Examiner has cited Downs, et al., February 1995, Development, Vol. 121, pp. 407-716 in a subsequent rejection as demonstrating "attachments of the allantois to the chorion is part of vasculogenesis . . . ." Applicants respectfully assert that the Examiner has misread Applicants' publication. Applicants enclose a Declaration from the author of the publication and inventor of the above-identified application, Dr. Karen Downs, disclosing that fusion with the chorion is not required for allantoic vascularization.

All claims are rejected as indefinite on the ground that the metes and bounds of what Applicants considered a "cultured allantoic explant" cannot be determined.

Applicants have substituted the phrase "allantoic tissue."

The claims have been rejected on the grounds that the metes and bounds of what Applicants consider "applying a test compound to a cultured allantoic explant" cannot be determined. Applicants have clarified that the allantoic tissue is treated with a compound. Applicants do not mean to encompass a situation wherein allantoic cells are contacted with recipient embryo.

All claims are rejected as missing at least one essential step. Applicants have now submitted new claims 28 and 29 to clarify the steps of the invention.

Claim 26 is rejected as indefinite. Applicants have cancelled claim 26.

§ 102 Rejections

All claims are rejected under 35 U.S.C. § 102(b) as anticipated by Downs, et al., 1995. In the third paragraph on page 11 of the Office Action, the Examiner characterizes Downs as teaching:

> In addition, Downs taught isolating whole or half allantoises from embryos and transplanting the allantois into a donor embryo (paragraph bridging pages 408 and 409) which is equivalent to "preparing an isolated allantois culture" because the allantois tissue is isolated from embryos and the cells are reproducing and because the allantois is cultured in another embryo *in ovo*. The transplanted allantois were observed for attachment to the chorion (page 40, column 1, first paragraph). Attachment of the allantois to the chorion is part of vasculogenesis because attachment to the

> allantois to the chorion is required for the allantois to become vascularized (page 407, column 2, 5 lines from the bottom) and become the umbilical chord. Therefore, observing attachment of the allantois to the chorion is equivalent to observing vasculogenesis. (underlining added)

Applicants respectfully disagree with the Examiner's characterization of the Downs, et al. article. Applicants have enclosed a Declaration by Dr. Karen Downs clarifying the observations made in the 1995 paper. (Dr. Downs signed a faxed copy of the Declaration. As a courtesy to the Examiner, Applicants have enclosed an identical, but unsigned, original copy of the Declaration.)

Dr. Downs disagrees that observing attachment of the allantois to the chorion is equivalent to observing vasculogenesis. As commented on above, Dr. Downs disagrees that attachment of the allantois to the chorion is necessary for vascularization or vasculogenesis.

The Examiner has asserted that the labeling of the allantoises in Downs, et al., 1995 with [^{3}H]methyl thymidine is equivalent to applying a test compound to a cultured allantoic explant. As emphasized in Dr. Downs' Declaration, no vascularization was observed. Dr. Downs and co-workers were simply observing the growth of the tissue.

Applicant believes the claims to be allowable and respectfully request a Notice of Allowance. A Petition and Fee for One Month Extension of Time is enclosed. If further fees are necessary, please charge Deposit Account 17-0055.

Respectfully submitted,

Karen M. Downs

January 7, 2002

By: Jean C. Baker

Jean C. Baker
Reg. No. 35,433
Quarles & Brady LLP
411 East Wisconsin Avenue
Milwaukee, WI 53202-4497
(414) 277-5709

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Karen M. Downs
Serial No.:	09/336,103
Filed:	June 18, 1999
For:	CHIMERIC MAMMALIAN ALLANTOIS
Group Art Unit:	1633
Examiner:	M. Wilson

Commissioner For Patents
Washington, D.C. 20231

MARKED UP VERSION OF THE CLAIMS AND SPECIFICATION

---

27. (Amended) The method of claim 25 wherein the test compound is a protein [gene product].

At page 1, line 10, please add: --This invention was made with United States government support awarded by the following agencies: NIH HD36847. The United States has certain rights in this invention.--

Please delete the paragraph on page 1 under "Cross Reference to Related Applications" and insert:

This application claims priority to U.S. Serial No. 08/838,384 --(abandoned)--, filed April 8, 1997, U.S. Serial No. 60/015,066, filed April 9, 1996 and to U.S. Serial No. 60/118,764, filed February 5, 1999. "Serial Nos. 60/015,066, 08/838,384 and 60/118,764 are herein incorporated by reference."

At page 1, lines 18 - 25 and page 2, lines 1 - 8, please delete the paragraph and insert:

During its early development, the murine allantois consists of an inner core of mesoderm and an outer layer of squamous epithelium referred to as a mesothelium. The allantois undergoes two major developmental processes: (i) maturation and fusion with the chorion to become the umbilical component of the chorioallantoic placenta, and (ii) vascularization, forming an artery and a vein that permit within the chorionic disk the exchange of nutrients, metabolic wastes and gases with the mother during fetal gestation (K.M. Downs and R.L. Gardner, Development 121:407-416, 1995; K.M. Downs and C. Harmann, Development 124:2769-2780, 1997; K.M. Downs, et al., The Murine Allantois. In Current Topics in Developmental Biology (eds. R. Pedersen and G. Schatten). New York: Academic Press. 39:1-33, 1998; K.M. Downs, et al., Development, submitted, 1999; K.M. Downs, supra, 1998).

At page 15, lines 3 - 27 and page 16, lines 1 - 2, please delete the paragraph and insert:

The mouse is an ideal model system for the study of umbilical development for several reasons. First, formation of the placenta occurs on schedule in whole embryo culture of living mouse conceptuses (K.M. Downs and R.L. Gardner, supra, 1995; K.M. Downs, et al., supra, 1998; K.M. Downs, et al., supra, 1999). Second, the allantois, precursor of the umbilical cord, is

particularly amenable to manipulation *in vitro* and can be isolated free of contamination from the conceptus (K.M. Downs and R.L. Gardner, supra, 1995; K.M. Downs and C. Harmann, supra, 1997; K.M. Downs, et al., supra, 1998; K.M. Downs, et al., supra, 1999; reviewed in K.M. Downs, supra, 1998). Third, transgenic mouse technology has enabled the identification of genes involved in formation of the placenta, either because its two major components, the allantois and the chorion, do not unite in the mutant mice (G.C. Gurtner, et al., Genes and Dev. 9:1-14, 1995; L. Kwee, et al., Development 121:489-503, 1995; J.T. Yang, et al., Development 121:549-560, 1995) or because vasculogenesis has not occurred in the umbilicus (R.J. Akhurst, et al., Development 108:645-656, 1990; M.C. Dickson, et al., Development 121:1845-1854, 1995; F. Shalaby, et al., Nature 376:62-66, 1995). Thus, the mouse is an ideal system in which to elucidate the genetic control of major developmental processes. There exists no other mammalian model at this time that exhibits all of these significant strengths.

At page 16, lines 15 - 28 and page 17, lines 1 - 11, please delete the paragraph and insert:

In the course of our studies, we demonstrated that when allantoises are removed from headfold-stage conceptuses (approximately 8.0 days postcoitum) and

cultured under relatively simple conditions in isolation, they rapidly undergo reproducible and stereotypic vasculogenesis (K.M. Downs, et al., supra, 1998 [illegible] 1999). With feeding, the allantoic vasculature is maintained and remodeled for up to 3 days. The cultured explants consist of at least three cell lineages, endothelial, mesothelial, and mesenchymal, all of which are normally found in intact allantoises. Further, correct topographical relations between at least two of these lineages, the endothelial and mesothelial cells, are maintained in the explants. Moreover, cells from explanted cultured allantoises can be returned to developmentally-equivalent host allantoises where they correctly colonize appropriate cell types. Lastly, one of the explanted cell populations, the mesenchymal cells, can take up and express exogenous DNA. On the basis of our findings, we propose that the murine allantois will be a powerful and extremely valuable model system for at least two novel applications (Method 1 and Method 2), described below:

At page 20, lines 3 - 28 and page 21, lines 1 - 6, please delete the paragraph and insert:

Vascular Endothelial Growth Factor (VEGF) is expressed in the allantoic mesothelium (D.J. Dumont, et al., Dev. Dyn. 203:80-92, 1995) before spreading into the

core (K.M. Downs, unpublished data). We have demonstrated that culture of allantoic explants in high rat serum (20-50% rat serum) is optimal for the formation of blood vessels (K.M. Downs, et al., supra, 1999, in revision). Culture of explants in low serum (fetal calf serum, FCS, 5-10%) favors formation of angioblasts, as revealed by expression of Flk-1 and Flt-1, early markers of angioblasts, but not their conversion into nascent blood vessels (K.M. Downs, et al., supra, 1999, in revision). Moreover, despite feeding, allantoises cultured in 5% FCS are typically devoid of vascular channels by 48 hours. By 72 hours, explants cultured in and fed 5% FCS at 24 hour intervals consist predominantly of mesenchymal cells. Increasing the concentration of FCS to 10-20% FCS results in partial maintenance of vascular channels for up to 72 hours, though significant breakdown of the channels is observed in about 87.5% of explants. Thus, a high concentration of some factor(s) must be required for both formation and maintenance of endothelial cells in allantoic explants. To test that possibility, recombinant VEGF (1-10 ng/ml culture medium) was added to explants at the start of culture in 5% FCS. Feeding at 24 hour intervals in the presence of Vascular Endothelial Growth Factor (2-10 ng/ml) resulted in formation of many vascular channels containing Flk-1 and Flt-1, and cell survival (78% cell retention compared

with 36% in untreated explants) whereas untreated explants or those treated with 1 ng/ml of VEGF were devoid of such channels.

At page 24, lines 26 - 28, page 25, lines 1 - 28, and page 26, lines 1 - 3, please delete the paragraph and insert:

Calcium phosphate-mediated transfection involves mixing DNA directly with $CaCl_2$ and a phosphate buffer to form a precipitate that is added to the cultured cells. This method achieves both transient and stable expression of DNA, the latter following integration of the transfected DNA into the host cell genome (M. Wigler, et al., Cell 16:777-785, 1979; M. Botchan, et al., Cell 20:143-152, 1980; S. Kato, et al., Mol. Cell. Biol. 6:1787-1795, 1986) or by autonomous replication in mini-chromosomal structures (D.H. Hamer, et al., Cell 17:725-735, 1979; D. DiMaio, et al., Proc. Natl. Acad. Sci. USA 79:4030-4034, 1982; R. Reeves, et al., Nucl. Acids Res. 13:3599-3615, 1985). As described above, allantoises will be removed and plated in individual wells of 24-well tissue culture dishes. One or more allantoises will be plated per well. Because CaP-mediated transfection requires that cells be 30-60% confluent, allantoises will be cultured for 12 hours, which is ample time for them to flatten out and spread somewhat on the bottom of the dish